SYNTHESIS, ANTI-OXIDANT AND ANTI-BACTERIAL PROPERTIES OF DIETHYL (4-FLOURO-3-NITRO PHENYLAMINO) (SUBSTITUTED PHENYL) METHYL PHOSPHONATES

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ABSTRACT: A new class of α–aminophosphoantes 4a-j have been synthesized by condensation of imines 3a-j with dialkyl phosphite under catalyst free conditions in dry toluene at reflux conditions via pudovik reaction in high yields. All the title compounds were confirmed by physico-spectral analysis. All the title compounds were screened for antioxidant properties by radical scavenging methods such as 1,1-diphenyl-2-picryl hydrazyl (DPPH), hydroxyl radical scavenging method and lipid peroxidation. They exhibited potent *in vitro* antioxidant activity dose dependently. Their bioassay showed them to possess significant antibacterial activity.

Key Words: α-Aminophosphonates; Pudovik reaction; Spectral analysis; *in vitro* antioxidant activity; antibacterial activity.

INTRODUCTION

Aminophosphonic acid derivatives constitute an important class of organophosphorus compounds on account of their versatile biological activity.[1] They are considered to be structural analogues of amino acids and transition state mimics of peptide hydrolysis. The general low mammalian toxicity of these compounds made them attractive for use in agriculture and medicine.[2-4] α-aminophosphonates were found too be effective in inhibiting test-tube growth of plasmodium falciparum, the parasite that causes malaria.[5] It has the same effect as related types of single-celled parasites such as Toxoplasma and Cryptosporidium that cause opportunistic infections in AIDS patients.[6] α-aminophosphonic acids have been found to act as inhibitors of specific enzymes as HIV protease, thrombin and human collagenase and to suppress the growth of various tumors and viruses.[7,8] Moreover, some aminophosphonic acids inhibit bone resorption, delay the progression of bone metastases, exert direct cytostatic effects on a variety of human tumor cells and have found clinical applications in the treatment of bone disorders and cancer.[9,10] Polymeric aminophosphonate analogues are used as bone seeking radiopharmaceuticals.[11] A series of new aminophosphonic acid derivatives of vin blastine (VCB) have been synthesized and evaluated for their *in vitro* and *in vivo* for antitumor activity.[12] In deed, it has been shown that some aminophosphonic acids can be used as anti-oxidants.[13] The pharmacological importance and utility of aminophosphonic acids derivatives have stimulated extensive studies on various aspects of chemistry: synthetic routes via pudovik reaction consisting of labile P-H addition to C=N is one of the main routes to α-aminophosphonates,[14,15] structural and spectral characterization and evaluation of their biological properties. In this work, we report the synthesis, spectroscopic characterization and *in vitro* antioxidant and antibacterial evaluation studies of new aminophosphonic acids diesters have been accomplished.

Antioxidants are predominantly studied as first-line therapy that protect organisms from deleterious effects induced by oxidative stress during metabolism.[16] In living organism, imbalance between reactive oxygen species (ROS) and antioxidants is referred as oxidative stress. Exogenous chemicals in food system and endogenous metabolic processes in human body produce highly reactive free radicals, especially oxygen derived ones. They are capable of oxidizing biomolecules and cause cell death and consequently cause tissue damage. Free radical oxidative processes also play a significant pathological role in causing human diseases. Many disease manifestations such as cancer, emphysema, cirrhosis, atherosclerosis and arthritis have been correlated with oxidative tissue damage. Also, excessive generation of reactive oxygen species (ROS) induced by various stimuli leads to variety of pathophysiological abnormalities such as inflammation, diabetes, genotoxicity and cancer. Search for active components that prevent or reduce the impact of oxidative stress on cell is a contemporary field.[17] In the present investigation, radical scavenging and antioxidative activity for the newly synthesized compounds are evaluated using three antioxidant methodologies.

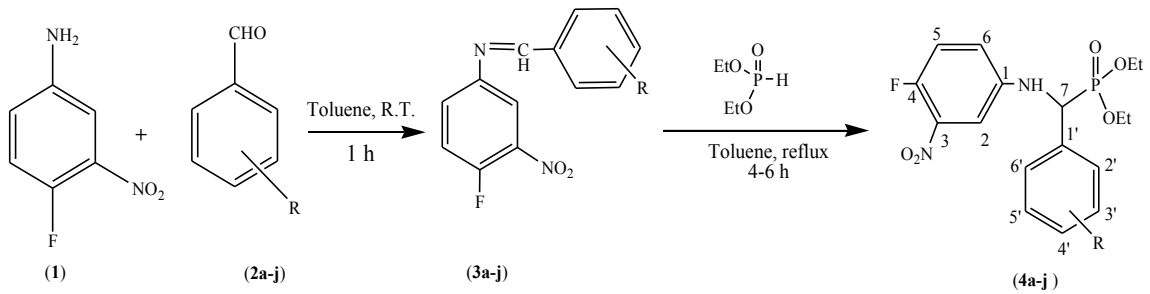
MATERIALS AND METHODS

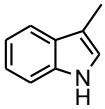
The chemicals procured were of commercial quality or chemically pure. All solvents were dried, deoxygenated and redistilled before use. The IR spectra (KBr pellets) were recorded on a Perkin-Elmer 283 unit. ^{1}H and ^{13}C spectra were recorded in DMSO-d_6 on a AMX 400 MHz spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C) with TMS as internal standard. ^{31}P NMR spectra were measured using 85% H_3PO_4 as external reference. Elemental analyses were performed by the Central Drug Research Institute, Lucknow, India. Mass spectra were recorded on Jeol 5 x 102 DA / 600 mass – spectrometer using argon / xenon (6 keV, 10 mA) as the fast atom bombardment (FAB) gas. Melting points were determined in open capillary tubes on Mel-temp apparatus, Tempo instruments, India and were uncorrected. The following abbreviations were used while presenting the NMR data s = singlet, d = double, t = triplet and m = multiplet.

RESULTS AND DISCUSSION

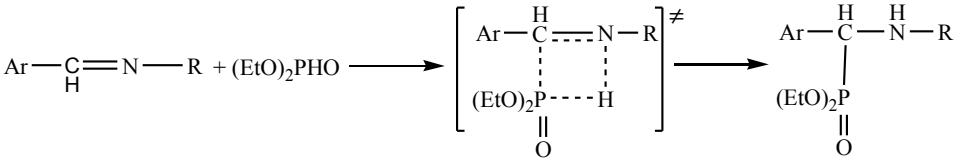
Imines 3a-j were obtained by condensation of the corresponding substituted aldehydes with 4-fluro-3-nitro aniline. Reaction of imines 3a-j with diethyl hydrogen phosphite via pudovik reaction in refluxing toluene in the absence of catalyst afforded the corresponding aminophosphonates 4a-j. (Scheme 1)

IR absorptions were observed in the regions 1217-1251, 742-758, and 3305-3438 cm^{-1} P=O, P-C(aliphatic) and for N-H respectively for 4a-j.[18,19] The aromatic hydrogens of the two benzene rings of α-aminophosphonates 4a-j showed a complex multiplet at δ 6.34-7.61. The P-C-H proton appeared as doublet at δ 4.70-5.29 ppm. The N-H proton showed a singlet at δ 4.70-6.03 for 4a-j respectively. ^{13}C NMR spectra for a few represented compounds showed carbon chemical shifts in their expected region. ^{31}P NMR chemical shifts occurred at δ 19.22-22.74.[19] The plausible pathway for this reaction appears to go via a highly organized four membered transition state in which the phosphonate (Scheme 2) hydrogen acting as an internal base and facilitates addition of P-H bond to form corresponding aminophosphonate.



Entry	R	Entry	R
2a&4a	4′ - F	2f&4f	
2b&4b	2′ - OH	2g&4g	4′ - OMe
2c&4c	5′ - Br, 2′ - OH	2h&4h	
2d&4d	4′ - Cl	2i&4i	2′ - OMe
2e&4e	2′ , 4′ - Cl	2j&4j	4′ - OH

Scheme 1 Synthesis of diethyl (4-flouro-3-nitro phenylamino) (substituted phenyl) methyl phosphonates



Scheme 2 Plausible pathway for the nucleophilic addition of diethyl phosphate to the imines via four membered transition state

The radical scavenging capacity of 4a-j was evaluated by 1,1-diphenyl-2-picryl hydrazyl(DPPH), anti lipid peroxidation and hydroxyl radical scavenging techniques. 4a, 4c and 4f showed appreciable antioxidant activity. Because both fluorine and -NO_2 substituents which affect the electron and hydrogen donating capacities, appears to be useful in inducing antioxidant activity. Since fluorine shows highly –ve inductive effect and –NO_2 is highly withdrawing moiety, thereby electron density around phosphonate moiety decrease and increases affinity towards oxygen derived free radicals and mobilizes ROS to be scavenged out of living system. Besides, due to presence of fluorine on aryl ring and ortho to hetero group increases lipophilicity can strongly polarize the parent molecule and seems to have profound effect on biological properties of title compounds. The presence of fluorine as a substituent also plays a determinant role in the inhibition of enzymes which are in importance in drug discovery such as mimicry and destabilization effects.[20]

ANTIOXIDANT ACTIVITY

1,1-Diphenyl-2-Picryl Hydrazyl (DPPH) Radical Scavenging Activity

The free radical scavenging activity of **4a-j, BHT (Butylated Hydroxyl Toulene)** and ascorbic acid were measured by the method of Blois (1958),[21] and the data are presented. The antioxidant activity of these compounds was expressed as IC_{50} (inhibitory concentration, 50%). DPPH forms a stable molecule on accepting an electron or a hydrogen and thus found application in the determination of radical scavenging and antioxidant activity.[22,23] In the case of aminophosphonates **4a** showed highest DPPH scavenging activity with IC_{50} of 4.20 mg/ml when compared with other compounds. The remaining compounds exhibited DPPH radical scavenging activity in the following order: **4b** (IC_{50} 5.00 mg/ml)› **4f** (IC_{50} 5.10 mg/ml) › **4c** (IC_{50} 5.30 mg/ml) **4g** (IC_{50} 5.90 mg/ml) **4d** (IC_{50} 6.20 mg/ml) **4j** (IC_{50} 7.80 mg/ml) **4e** (IC_{50} 8.40 mg/ml) **4i** (IC_{50} 9.40 mg/ml) **4h** (IC_{50} 9.70 mg/ml) and was significant (p-0.001) when compared with that of ascorbic acid and BHT (IC_{50} 5.40 mg/ml and 3.20 mg/ml).

$$\text{DPPH scavenged (\%)} = \frac{A_{cont} - A_{test}}{A_{cont}} \text{ X } 100$$

Where A_{cont} is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample.

Lipid Peroxidation Assay

Lipid peroxidation was induced by Fe^{2+} ascorbate complex system in rat red blood cells and estimated as thiobarbituric acid reacting substances (TBARS).[24] Experiments *in vitro* lipid peroxidation were carried out to clarify the mode of the protective effect of the aminophosphonates against oxidative stress-induced cell damage. The inhibition of lipid peroxidation is used as a model to elucidate antioxidant activity. According to the obtained results, **4a** significantly (p-0.001) inhibited the ferric ion plus ascorbic acid in rat red blood cells with IC_{50} of 3.80 mg/ml when compared with other compounds The remaining compounds exhibited lipid peroxidation scavenging activity in the following order: **4c** (IC_{50} 5.01 mg/ml) › **4f** (IC_{50} 5.20 mg/ml) › **4b** (IC_{50} 5.50 mg/ml) › **4j** (IC_{50} 5.70 mg/ml) › **4g** (IC_{50} › 5.80 mg/ml) › **4d** (IC_{50} 6.01 mg/ml) › **4e** (IC_{50} 6.50 mg/ml) › **4i** (IC_{50} 7.50 mg/ml) › **4h** (IC_{50} 7.80 mg/ml). The results are significant (p-0.001) when compared with that of ascorbic acid and BHT (IC_{50} 5.40 mg/ml and 3.20 mg/ml).

Hydroxyl Radical Scavenging Activity

It was carried out by measuring the competition between deoxyribose and the compounds that generate hydroxyl radicals from the Fe^{3+}/ascorbate/EDTA/H_2O_2 system. Attack of the hydroxyl radicals on deoxyribose led to formation of thiobarbituric acid-reactive substances (TBARS) which were measured by the method of Ohkawa *et al.* (1979).[25] The hydroxyl radical is the most reactive oxygen species (ROS) that attacks almost every molecule in the body and also leads to DNA damage in a cell. It initiates the peroxidation of cell membrane lipids[26,27] increases MDA levels which is cytotoxic, mutagenic and carcinogenic.[28] The compound **4a** showed significant hydroxyl radical scavenging activity with IC_{50} of 5.10 mg/ml when compared with other compounds.

The remaining compounds exhibited hydroxyl radical scavenging activity in the following order respectively: **4c** (IC_{50} 5.40 mg/ml) › **4b** (IC_{50} 5.70 mg/ml) › **4f** (IC_{50} 5.80 mg/ml) › **4j** (IC50 7.40 mg/ml) › **4g** (IC_{50} 7.80 mg/ml) › **4d** (IC_{50} 8.01 mg/ml) › **4e** (IC_{50} 9.60 mg/ml) › **4c** (IC_{50} nil) › **4d** (IC_{50} nil) and was significant (p-0.001) when compared to ascorbic acid and (IC_{50} 5.40 mg/ml and 3.80mg/ml).

$$\text{OH scavenged } (\%) = \frac{A_{cont} - A_{test}}{A_{cont}} \text{X} 100$$

Where A_{cont} is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample.

Antibacterial Activity

For bioassays of compounds 4a-j (Table 1), a suspension of approximately 1.5 x 10^8 bacterial cells/ml in sterile normal saline was prepared as described by Forbes et al.[29] Wells in the agar medium were punched and filled with the title compounds at concentration of 100µg in each well. The plates were incubated for 24h at 37^{O}C for test bacteria. Ciprofloxacin was employed as standard antibiotic to compare the activity of test compounds. 4c had emerged as a lead compound with DIZ 14.86±0.14 µg/well. Minimum inhibitory concentration (MIC) was determined for the compounds 4a-j (Table 2) by micro broth dilution method[30] that showed total growth inhibition. 4f had emerged as a lead compound with MIC value of 119.01±0.87 µg/well. The compound concentration of 50 µg to 300 µg in steps of 20 µg was evaluated. The lowest concentration (highest dilution) of the culture that produced no visible signs of bacterial growth (no turbidity) when compared with the control tubes were regarded as MIC.

General Procedure for Preparation of Diethyl (4-flouro-3-nitro phenylamino) (4-flouro phenyl) methyl phosphonate (4a)

A mixture of 4-fluoro-3-nitro aniline (1) (0.62 g, 0.004mol) and 4-fluorobenzaldehyde (0.42 mL, 0.005 mol) was stirred in anhydrous toluene (15ml) at room temperature for 1 h. Diethyl phosphite (0.50 mL, 0.004 mol) in anhydrous toluene (15 mL) was added dropwise. Stirring was continued at room temperature for another 0.5 h, after which the mixture was heated under reflux for 4-6 h. The reaction was monitored by TLC on silica gel using petroleum ether-ethylacetate (1:2 v/v). After completion of the reaction, the solvent was removed by rotaevaporator and the resulting residue was purified by column chromatography on silicagel (100-200 mesh and ethyl acetate-hexane) as eluent to afford pure α-aminophosphonate (4a).

The Other compounds 4b-j were prepared by employing the above described procedure for compound 4a.

Diethyl (4-flouro-3-nitro phenylamino) (4-flouro phenyl) methyl phosphonate (**4a**). Yellow crystals: Yield was found to be 82%, m.p. 160-162 ºC. IR (KBr) ν cm^{-1}: 3281, 1228, 753. ^{1}H-NMR (DMSO-d_6): δ 6.68-7.46 (m, 7H, Ar-H), 6.03 (s, 1H, NH), 4.72 (d, J = 24 Hz, 1H, P-C- H), 3.70-4.21(m, 4H, 2×OCH_2), 1.32 (t, J = 7Hz, 3H, P-O-CH_2- CH_2), 1.14 (t, J = 7 Hz, 3H, P-O-CH_2- CH_3). ^{13}C-NMR (DMSO-d_6): δ 148.20 (C-1), 110.25 (C-2), 132.07 (C-3), 145.95 (C-4), 114.76 (C-5), 117.85 (C-6), 54.41 (C-7), 130.37 (C-1'), 129.57 (C-2'), 115.99 (C-3'), 163.65 (C-4'), 115.17 (C-5'), 129.53 (C-6'), 63.82 (d, J = 7.5 Hz ,OCH_2), 16.43 (d, J = 6.25 Hz, O-CH_2- CH_2). ^{31}P-NMR (DMSO-d_6): δ 22.61. FAB MS: m/z (%) 439 (M+K). Anal. Calcd. for $C_{17}H_{19}F_2N_2O_5P$: C, 51.01; H, 4.78; N, 7.00. Found C, 50.9; H, 74; N, 6.95.

Diethyl (4-flouro-3-nitro phenylamino) (2-hydroxy phenyl) methyl phosphonate (**4b**). Yellow crystals: Yield was found to be 80%, m.p. 137-139 ºC. IR (KBr) ν cm^{-1}: 3274, 1217, 751. ^{1}H-NMR (DMSO-d_6): δ 6.73-7.32 (m, 7H, Ar-H), 4.94 (s, 1H, NH), 5.14 (d, J = 23.5 Hz, 1H, P-CH), 3.81-4.20 (m, 4H, 2×OCH_2), 1.31 (t, J = 7Hz, 3H, P-O-CH_2- CH_3), 1.15 (t, J= 7 Hz, 3H, P-O-CH_2- CH_3). ^{13}C-NMR (DMSO-d_6): δ 148.21 (C-1), 109.88 (C-2), 132.08 (C-3), 145.84 (C-4), 118.43 (C-5), 120.61 (C-6), 51.93 (C-7), 120.70 (C-1'), 155.23 (C-2'), 117.25 (C-3'), 128.95 (C-4'), 120.61 (C-5'), 129.88 (C-6'), 64.03 (d, J = 7.5 Hz ,OCH_2), 16.15 (d, J = 6.25 Hz,O-CH_2- CH_3). ^{31}P-NMR (DMSO-d_6): δ 22.61. FAB MS: m/z (%) 398 (M+1). Anal. Calcd. for $C_{17}H_{20}FN_2O_6P$: C, 51.26; H, 5.06; N, 7.03. Found C, 51.15; H, 5.02; N, 6.98.

Diethyl (4-flouro-3-nitro phenylamino) (5-bromo-2-hydroxy phenyl) methyl phosphonate (**4c**). Yellow crystals: Yield was found to be 72%, m.p. 85-86 ºC. IR (KBr) ν cm^{-1}: 3280, 1227, 743. ^{1}H-NMR (DMSO-d_6): δ 6.43-7.53 (m, 6H, Ar-H), 4.84 (s, 1H, N), 4.82 (d, J = 24 Hz, 1H, P-CH), 3.74-4.21 (m, 4H, 2×OCH_2), 1.34 (t, J = 7Hz, 3H, P-O-CH_2- CH_3), 1.14 (t, J= 7 Hz, 3H, P-O-CH_2- CH_3). ^{31}P-NMR (DMSO-d_6): δ 21.08. Anal. Calcd. for $C_{17}H_{19}BrFN_2O_6P$: C, 42.79; H, 4.01; N, 5.87. Found C, 42.68; H, 3.97; N, 5.82.

Diethyl (4-flouro-3-nitro phenylamino) (4-chloro phenyl) methyl phosphonate (**4d**). Yellow crystals: Yield was found to be 74%, m.p. 177-179 ºC. IR (KBr) ν cm^{-1}: 3280, 1225, 750. ^{1}H-NMR (DMSO-d_6): δ 6.66-7.45 (m, 7H, Ar-H), 5.49 (s, 1H, NH), 4.70 (d, J = 23.5 Hz, 1H, P-CH), 3.69-4.19 (m, 4H, 2×OCH_2), 1.33 (t, J = 7.2 Hz, 3H, P-O-CH_2- CH_3), 1.14 (t, J = 7 Hz, 3H, P-O-CH_2- CH_3). ^{13}C-NMR (DMSO-d_6): δ 148.14 (C-1), 110.49 (C-2), 132.04 (C-3), 143.90 (C-4), 114.63 (C-5), 117.63 (C-6), 55.68 (C-7), 134.28 (C-1'), 129.23 (C-2'), 129.01 (C-3'), 133.35 (C-4'), 129.01 (C-5'), 129.23 (C-6'), 63.74 (d, J = 6.2 Hz ,OCH_2), 16.45 (d, J = 5.0 Hz,O-CH_2- CH_3). ^{31}P-NMR (DMSO-d_6): δ 20.76. FAB MS: m/z (%) 416, 418(M+2). Anal. Calcd. for $C_{17}H_{19}ClFN_2O_5P$: C, 48.99; H, 4.60; N, 6.72. Found C, 48.87; H, 4.55; N, 6.68.

Diethyl (4-flouro-3-nitro phenylamino) (2,4-dichloro phenyl) methyl phosphonate (**4e**). Yellow crystals: Yield was found to be 70%, m.p. 164-166 ºC. IR (KBr) ν cm^{-1}: 3283, 1222, 749. ^{1}H-NMR (DMSO-d_6): δ 6.67-7.60 (m, 6H, Ar-H), 4.75 (s, 1H, NH), 5.29 (d, J = 23.5 Hz, 1H, P-CH), 3.72-4.27 (m, 4H, 2×OCH_2), 1.36 (t, J = 7.2 Hz, 3H, P-O-CH_2- CH_3), 1.13 (t, J = 7 Hz, 3H, P-O-CH_2- CH_3). ^{13}C-NMR (DMSO-d_6): δ 148.19 (C-1), 109.86 (C-2), 132.06 (C-3), 145.90 (C-4), 114.61 (C-5), 117.63 (C-6), 55.61 (C-7), 134.26 (C-1'), 129.01 (C-2'), 129.33 (C-3'), 133.33 (C-4'), 129.23 (C-5'), 129.02 (C-6'), 63.76 (d, J = 6.2 Hz ,OCH_2), 16.42 (d, J = 5.0 Hz,O-CH_2- CH_{33}) ^{31}P-NMR (DMSO-d_6): δ 20.29. Anal. Calcd. for $C_{17}H_{18}Cl_2FN_2O_5P$: C, 45.25; H, 4.02; N, 6.21. Found C, 45.14; H, 3.98; N, 6.16.

Table 1 Antibacterial Activity (Diameter of Zone of Inhibition in mm) of Compounds 4a-j (100 µg/ml)

Diameter Inhibitory Zone(100 µg/well)

Bacteria	*Ciproflaxacin* (5µg/well)	4a	4b	4c	4d	4e	4f	4g	4h	4i	4j
Staphylococcus aureus	24.23 ± 1.19	12.19 ± 0.96	11.24 ± 1.76	14.86 ± 0.14	8.63±2.31	9.38 ± 1.10	10.23 ± 2.77	8.02 ± 0.16	12.84 ± 0.16	9.02 ± 1.44	11.56 ± 1.42
Bacillus faecalis	22.69 ±0.09	12.86 ± 1.14	10.45 ± 3.55	13.58 ± 1.42	7.96±3.22	8.96 ± 2.04	11.82 ± 1.18	7.95 ± 0.23	12.06 ± 1.94	8.77 ± 1.71	10.95 ± 2.05
Escherichia coli	26.35±1.12	14.18 ±0.62	13.54 ±0.46	16.03 ±0.24	8.88±0.9	12.04 ± 0.82	14.13 ±0.35	10.34 ±0.14	14.45 ±0.11	12.08 ± 0.08	13.94 ± 0.06
Pseudomonas aeruginosa	24.21±2.10	13.84 ±0.16	12.68 ±1.32	15.96 ±1.02	9.22±2.33	11.64 ±0.36	13.54 ±0.46	11.02 ±0.08	13.86 ±0.14	12.38 ± 0.12	12.32 ± 1.68
Salmonella typhimurium	21.53±3.25	12.98 ± 1.02	11.98 ± 2.02	13.98 ± 2.97	10.12±1.3	10.95 ± 1.05	11.86 ± 2.14	8.94 ± 1.06	11.95 ± 2.05	10.56 ± 1.44	11.62 ± 2.38
Klebsiella pneumoniae.	22.69±4.23	13.62 ± 0.38	12.08 ± 1.92	14.98 ± 1.02	8.6±1.3	11.83 ± 0.17	12.35 ± 1.65	9.32 ± 0.68	13.26 ± 0.74	11.25 ± 0.75	12.65 ± 1.35

Table 2 MIC of Compounds 4a-j (µg/ml).

Minimum Inhibitory Concentration(MIC µg/ml)

Bacteria	Cf	4a	4b	4c	4d	4e	4f	4g	4h	4i	4j
Staphylococcus aureus	6.12±0.86	130.11±1.23	136.20±3.66	120.23±1.63	218.20±3.25	145.55±3.11	119.01±0.87	280.55±036	131.66±2.54	153.77±1.61	129.55±36
Bacillus faecalis	5.54±0.23	136.08±0.33	142.11±2.88	102.33±.2.3	196.23±5.36	151.02±0.88	109.31±0.99	265.45±0.69	147.88±4.15	161.10±0.55	133.96±0.55
Escherichia coli	7.51±1.20	128.23±1.56	132.31±3.22	82 .96±1.22	178.96±9.36	162.14±2.55	121.78±0.9	277.33±3.01	144.071±1.55	169.38±3.69	141.10±1.8
Pseudomonasaeruginosa	6.98±1.01	130.19±1.94	128.10±1.66	96.01±0.56	156±2.3	174.77±0.63	125.31±3.55	281.88±0.59	152.32±2.11	148.27±0.58	138.09±0.55
Salmonella typhimurium	5.69±0.36	126.66±2.15	137.09±0.99	79.55±0.21	166.57±2.33	144.01±2.33	114.02±4.01	268.21±2.36	138.66±1.32	152.32±5.10	122.88±0.96
Klebsiella pneumoniae.	4.88±0.58	138.02±3.21	122.55±1.77	114.66±2.33	185.36±1.20	139.22±3.17	118.03±3.22	271.11±1.02	129.77±0.09	141.61±1.22	126.22±0.32

Diethyl (4-flouro-3-nitro phenylamino) (furan-2-yl) methyl phosphonate (**4f**). Yellow crystals: Yield was found to be 68%, m.p. 174-176 ºC. IR (KBr) ν cm^{-1}: 3305, 1241, 758. ^{1}H-NMR (DMSO-d_6): δ 6.34-7.39 (m, 5H, Ar-H), 7.29 (s, 1H, Ar-CH), 5.28 (s, 1H, NH), 4.88 (d, J = 23.5 Hz, 1H, P-C<u>H</u>), 3.87-4.25 (m, 4H, 2×OCH_2), 1.32 (t, J = 7.2 Hz, 3H, P-O-CH_2- CH_3), 1.22 (t, J = 7 Hz, 3H, P-O-CH_2-CH_3). ^{13}C-NMR (DMSO-d_6): δ 147.57 (C-1), 109.55 (C-2), 132.01 (C-3), 143.14 (C-4), 118.81 (C-5), 120.33 (C-6), 50.80 (C-7), 149.61 (C-1'), 109.39 (C-2'), 110.92 (C-3'), 142.88 (C-4'), 63.65 (d, J = 7.5 Hz ,OCH_2), 16.40 (d, J = 5.0 Hz, O-CH_2- CH_3). ^{31}P-NMR (DMSO-d_6): δ 19.22. FAB MS: m/z (%) 356 (M+1). Anal. Calcd. for $C_{17}H_{19}ClFN_2O_5P$: C, 48.39; H, 4.87; N, 7.52. Found C, 48.28; H, 4.83; N, 7.47.

Diethyl (4-flouro-3-nitro phenylamino) (4-methoxy phenyl) methyl phosphonate (**4g**). Yellow crystals: Yield was found to be 78%, m.p. 182-184 ºC. IR (KBr) ν cm^{-1}: 3290, 1227, 742. ^{1}H-NMR (DMSO-d_6): δ 6.54-7.61 (m, 7H, Ar-H), 5.61 (s, 1H, NH), 5.12 (d, J = 23.5 Hz, 1H, P-CH), 3.76 (s, 3H, Ar-OCH_3), 3.84-4.19 (m, 4H, 2×OCH_2), 1.38 (t, J = 7.2 Hz, 3H, P-O-CH_2-C<u>H</u>$_3$), 1.12 (t, J = 7 Hz, 3H, P-O-CH_2-C<u>H</u>$_3$). ^{31}P-NMR (DMSO-d_6): δ 22.35. Anal. Calcd. for $C_{18}H_{22}FN_2O_6P$: C, 52.43; H, 5.38; N, 6.79. Found C, 52.32; H, 5.34; N, 6.74.

Diethyl (4-flouro-3-nitro phenylamino) (1H-indol-4-yl) methyl phosphonate (**4h**). Yellow crystals: Yield was found to be 66%, m.p. 124-126 ºC. IR (KBr) ν cm^{-1}: 3320, 1251, 747. ^{1}H-NMR (DMSO-d_6): δ 7.02-7.68 (m, 8H, Ar-H), 9.87(s, 1H, N<u>H</u> Hetero), 4.85 (s, 1H, NH), 4.55 (d, J = 23.5 Hz, 1H, P-C<u>H</u>), 3.60-3.90 (m, 4H, 2×OCH_2), 1.52 (t, J = 7.0 Hz, 3H, P-O-CH_2- CH_3), 1.12 (t, J = 6.5 Hz, 3H, P-O-CH_2- CH_3). ^{31}P-NMR (DMSO-d_6): δ 24.28. Anal. Calcd. for $C_{19}H_{20}FN_3O_5P$: C, 54.29; H, 4.80; N, 10.00. Found C, 54.18; H, 4.76; N, 9.95.

Diethyl (4-flouro-3-nitro phenylamino) (2-methoxy phenyl) methyl phosphonate (**4i**). Yellow crystals: Yield was found to be 76%, m.p. 180-182 ºC. IR (KBr) ν cm^{-1}: 3314, 1230, 755. ^{1}H-NMR (DMSO-d_6): δ 6.51-7.54 (m, 7H, Ar-H), 5.52 (s, 1H, NH), 4.92 (d, J = 23.5 Hz, 1H, P-CH), 3.74 (s, 3H, Ar-OCH_3), 3.79-4.16 (m, 4H, 2×OCH_2), 1.36 (t, J = 7.2 Hz, 3H, P-O-CH_2- CH_3), 1.16 (t, J = 6.5 Hz, 3H, P-O-CH_2- CH_3). ^{31}P-NMR (DMSO-d_6): δ 22.74. Anal. Calcd. for $C_{18}H_{22}FN_2O_6P$: C, 52.43; H, 5.38; N, 6.79. Found C, 52.31; H, 5.34; N, 6.75.

Diethyl (4-flouro-3-nitro phenylamino) (4-hydroxy phenyl) methyl phosphonate (**4j**). Yellow crystals: Yield was found to be 72%, m.p. 126-128 ºC. IR (KBr) ν cm^{-1}: 3338, 1237, 754. ^{1}H-NMR (DMSO-d_6): δ 6.69-7.59 (m, 7H, Ar-H), 4.76 (s, 1H, NH), 5.27 (d, J = 23.5 Hz, 1H, P-CH), 3.71-4.19 (m, 4H, 2×OCH_2), 1.37 (t, J = 7.0 Hz, 3H, P-O-CH_2- CH_3), 1.12 (t, J = 7.0 Hz, 3H, P-O-CH_2-C<u>H</u>$_3$). ^{31}P-NMR (DMSO-d_6): δ 22.64. Anal. Calcd. for $C_{17}H_{20}FN_2O_6P$: C, 51.26; H, 5.06; N, 7.03. Found C, 51.14; H, 5.01; N, 6.97.

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